

09/599,877

Docket No. TIBO-0011/VIP0007

In the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application

Listing of Claims:

1. (Currently Amended) A method for determining the AZT susceptibility of a test HIV-1 RT enzyme, comprising:

a) providing a reaction well with the following reaction components comprising:

- i. at least one template for an HIV-1 RT enzyme;
- ii. at least one primer;
- iii. at least one detectable dNTP substrate;
- iv. AZT; and
- v. at least one ~~ribonucleotide compound~~ chosen from ATP at a final concentration in the range of from about 1.5 mM to about 6 mM in the reaction well or GTP, or at least one pyrophosphate at about the physiological concentration in the reaction well;

b) performing an enzymatic kinetics assay ~~that permits the measurement of multiple chain termination events by adding to the reaction well the test HIV-1 RT enzyme;~~

~~wherein said test HIV-1 RT enzyme incorporates the at least one detectable dNTP substrate or AZT into the 3' end of the at least one primer forming at least one new DNA strand that is complementary to and bound to said at least one template;~~

c) measuring the amount of the detectable dNTP substrate bound to the template;

d) repeating steps a) through c), replacing the test HIV-1 RT enzyme with a control HIV-1 RT enzyme that is known to be susceptible to AZT inhibition; and

e) comparing the amount of the detectable dNTP substrate measured from step c) with that from step d);

wherein the test HIV-1 RT enzyme is identified to be AZT resistant when the amount of the detectable dNTP substrate measured from step c) is more than that from step d); and the test HIV-1 RT enzyme is identified to be AZT susceptible when the amount of the detectable dNTP substrate measured from step c) is less than or equal to that from step d).

2. (Original) The method of claim 1, wherein the template is bound to the reaction well and is chosen from poly-rA or a heteropolymer RNA or DNA.
3. (Original) The method of claim 1, wherein the primer is chosen from oligo-dt or a primer that is complementary to the heteropolymer template.
4. (Original) The method of claim 1, wherein the detectable dNTP substrate is chosen from a radioactive labeled dNTP.
5. (Original) The method of claim 1, wherein the detectable dNTP substrate is capable of being detected by fluorescence, luminescence, or absorption spectrometry.
6. (Original) The method of claim 1, wherein the detectable dNTP substrate binds to an optical tracer or a radioactive labeled tracer.
7. (Original) The method of claim 6, wherein the optical tracer is capable of being detected by fluorescence, luminescence, or absorption spectrometry.
8. (Original) The method of claim 6, wherein the detectable dNTP precursor is bromo-deoxyuridine-triphosphate.
9. (Currently Amended) The method of claim ~~7~~8, wherein the optical tracer is a monoclonal anti-BrdU antibody, conjugated to alkaline phosphatase.
- 10 – 19. (Cancelled)
- 10 ~~20~~. (Currently Amended) A method for identifying at least one mutation in an HIV-1 RT enzyme that increases or decreases the AZT susceptibility of the HIV-1RT enzyme, comprising:
 - a) providing a reaction well with the following reaction components comprising:
 - i. at least one template for an HIV-1 RT enzyme;
 - ii. at least one primer;
 - iii. at least one detectable dNTP substrate;

iv. AZT; and

v. at least one ribonucleotide compound chosen from ATP at a final concentration in the range of from about 1.5 mM to about 6 mM in the reaction well or GTP, or at least one pyrophosphate at about the physiological concentration in the reaction well;

- b) performing an enzymatic kinetics assay ~~that permits the measurement of multiple chain termination events by adding to the reaction well a HIV-1 RT enzyme;~~
~~wherein said HIV-1 RT enzyme incorporates the at least one detectable dNTP substrate or the AZT into the 3' end of the at least one primer forming at least one new DNA strand that is complementary to and bound to said at least one template;~~
- c) determining RT activity by measuring the amount of the detectable dNTP substrate bound to the template;
- d) repeating steps a) through c), replacing the HIV-1 RT enzyme with a mutant HIV-1 RT enzyme comprising at least one mutation, wherein the mutant HIV-1 RT enzyme is otherwise identical to the HIV-1 RT enzyme except for the at least one mutation; and
- e) comparing the amount of the detectable dNTP substrate measured from step c) with that from step d);
wherein the at least one mutation increases the AZT susceptibility of the HIV-1 RT enzyme when the amount of the detectable dNTP substrate measured from step c) is more than that from step d); and the at least one mutation decreases the AZT susceptibility of the HIV-1 RT enzyme when the amount of the detectable dNTP substrate measured from step c) is less than that from step d).

1/ ~~21~~. (Currently Amended) A method for rapid screening of mutations in an HIV-1 RT enzyme that increase or decrease the AZT susceptibility of the HIV-1 RT enzyme, comprising:

- a) providing an array of reaction wells, each reaction well with the following reaction components comprising:
 - i. at least one template for an HIV-1 RT enzyme;
 - ii. at least one primer;
 - iii. at least one detectable dNTP substrate;
 - iv. AZT; and
 - v. at least one ribonucleotide compound chosen from ATP at a final concentration in the range of from about 1.5 mM to about 6 mM in the reaction well or GTP, or at least one pyrophosphate at about the physiological concentration in the reaction well;
- b) performing an enzymatic kinetics assay ~~that permits the measurement of multiple chain termination events by adding to each reaction well a RT enzyme chosen from an HIV-1 RT enzyme or a mutant HIV-1 RT enzyme comprising at least one mutation, wherein the mutant HIV-1 RT enzyme is otherwise identical to the HIV-1 RT enzyme except for the at least one mutation;~~
~~wherein said HIV-1 RT enzyme incorporates the at least one detectable dNTP substrate or the AZT into the 3' end of the at least one primer forming at least one new DNA strand that is complementary to and bound to said template and wherein the HIV-1 RT enzyme is added to at least one reaction well of the array of reaction wells;~~
- c) in each reaction well measuring the amount of the detectable dNTP substrate bound to the template; and
- d) comparing the amount of the detectable dNTP substrate measured from the well containing the mutant HIV-1 RT enzyme with that from the well containing the HIV-1 RT enzyme;

wherein mutations that increase or decrease the AZT susceptibility of the HIV-1 RT enzyme are rapidly identified. .

12 ~~22~~. (Previously Presented) The method of claim 1, wherein the control RT enzyme in step d) is a wild-type HIV-1 RT enzyme.

13 ~~23~~. (Currently Amended) The method of claim ¹⁰~~20~~, further comprising the steps of:
f) repeating step d) of the method of claim ¹⁰~~20~~, omitting the at least one ribonucleotide compound chosen from ATP at a final concentration in the range of from about 1.5 mM to about 6 mM in the reaction well or GTP, or at least one pyrophosphate at about the physiological concentration in the reaction well;
g) comparing the amount of the detectable dNTP substrate measured from ¹⁰step f) with that from step c) of claim ¹⁰~~20~~, and with that from step d) of claim ¹⁰~~20~~;
wherein the at least one test mutation can be resistant to other HIV inhibitor(s) in addition to AZT when the amount of the detectable dNTP substrate measured from step f) is more than that from step c) of claim ¹⁰~~20~~, and is substantially the same as that from step d) of claim ¹⁰~~20~~.

14 ~~24~~ (New) The method of claim 1, wherein the ATP is at about the physiological concentration in the reaction well.

15 ~~25~~. (New) ¹⁴The method of claim ~~24~~, wherein the ATP is at a final concentration of about 3.2 mM in the reaction well.

16 ~~26~~. (New) The method of claim 1, wherein the pyrophosphate is at a final concentration of about 150 μ M in the reaction well.